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Functional properties of powdered β-lactoglobulin – cholecalciferol complexes

K. Szulc
Department of Food Engineering and Process Management, Faculty of Food Sciences,
Warsaw University of Life Sciences (SGGW-WULS), Poland;
E-mail: karolina_szulc@sggw.pl

A. Górska
Department of Chemistry, Faculty of Food Sciences,
Warsaw University of Life Sciences (SGGW-WULS), Poland;
E-mail: agata_górska@sggw.pl

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The objective of the present study was to assess the influence of the drying method on selected physical properties of powdered complexes of β-lactoglobulin – cholecalciferol (β-LG – vitamin D3) with addition of carbohydrates. Improved wettability and reduced hygroscopicity were observed for β-LG – vitamin D3 complexes with addition of carbohydrates. Powdered complexes of β-LG – vitamin D3 with/without addition of lactose/trehalose obtained by freeze-drying were characterised by a larger particle size and better wettability as compared to spray-dried powders. The stability of amorphous powders depends on the material composition and storage conditions. The results showed that addition of carbohydrates, lactose or trehalose had a similar water sorption but different crystallization properties. In the case of β-LG – vitamin D3 complexes, the crystallization of carbohydrates, especially trehalose crystallization, was delayed.

Introduction

β-lactoglobulin (β-LG) is a major whey protein possessing a nutritional value as well as functional characteristics [1–3]. Although the biological functions of the protein still remain elusive, some essential functions of β-LG, such as cholesterol lowering, modulation of the immune system, transport of retinol, fatty acid, and vitamin D and prevention of oxidative stress have been reported [3, 4]. Since β-lactoglobulin can be produced as a spray-dried powder, it is logical to market vitamin-fortified β-LG as a powder form not only for its convenience and versatile use in different food applications, but also for its shelf stability. During the drying process, heat tends to denature the protein and causes the dissociation of the complex, which can result in a low retention of vitamins. Certain sugars, such as lactose and trehalose, were found to stabilize whey protein during spray-drying [5–7]. According to Jouppila and Roos [8], proteins seem to limit or retard milk powder crystallization. In addition, lactose is known to be an efficient cryo-protector due to lactose–protein hydrogen bonds, which could also act to reduce crystallization kinetics [9].

Two drying methods (freeze-drying and spray-drying) were employed to compare the functional properties of obtaining these complexes. Freeze drying, also known as lyophilization, is sometimes preferred for unstable or heat-labile foods or when the product quality and structural integrity are critical. Freeze drying is generally considered as the best method for the production of high-quality dried products [10], but it suffers from high production costs, high energy consumption, and low throughputs [10, 11]. Spray drying is the dehydration process best suited for liquid food products with high initial moisture contents. The technical functionalities of a food powder, e. g., dispersability, wettability, and flowability, are known to be partly determined by the physical structure and surface composition of the powder [12, 13].

The aim of this study was to assess the influence of the drying method on selected physical properties of powdered complexes of β-lactoglobulin – vitamin D3 with lactose or trehalose addition.

Materials and methods

The materials used in the study were β-lactoglobulin (β-LG) (Davisco Foods International), cholecalciferol (vitamin D3) (Sigma-Aldrich), lactose (L) (Poch S.A.), trehalose (T) (Poch S.A.).

400 ml of β-lactoglobulin solution was prepared by gently adding distilled water into 8.6 g of the protein while stirring slowly to avoid heavy foaming. The mixture was kept at room temperature until a homogenous clear solution was formed. Then 0.36 g of cholecalciferol dissolved in 800 µl absolute ethanol was added into the solution to obtain the 2 : 1 molar ratio of vitamin D3 to protein. The solution was incubated at 40 °C for 2 h according to the method described by Kontopidis et al. (2004). Additionally, complexes containing carbohydrates were prepared by adding

lactose/trehalose to the protein solution in a weight ratio 5 : 1. The formulations were kept until the lactose/trehalose was dissolved, and then dried.

The β-LG – vitamin D3 complexes were spray-dried in a laboratory spray-dryer (Anhydro). The inlet air temperature was 120 °C, and the liquid feed to the dryer was 51.4 ml/min. Complexes were also placed in aluminum trays and frozen at -40 °C for 4 h in a shock freezer (Irinox). The material was then dried (24 h) in an Gamma 1–16 LSC freeze-dryer (Christ) under a vacuum of 63 Pa and with the heating plate temperature of 30 °C.

The physical properties analysed were water activity, particle size, insolubility index, wettability, and adsorption kinetics. The water activity of powdered complexes was measured with a water activity meter HydroLab C1 (Rotronic). The mean diameter of particles was determined with a solid particle size analyzer in air AWK-V97 (Kamika). The laser radiation stream in infrared not only identifies the size of particles, but also renders counting them precisely in the entire measuring range possible. An electric impulse corresponds to each particle. The impulse is proportional to the particle size. The set of particles is initially measured with the division into 4096 channels and converted (calibrated) into 256 size classes, which are available to a user.

The insoluble index is a measure for the ability of a powder to dissolve in water. It is defined as the volume of sediments per ml after centrifuging [14]. The wettability of the powder was determined according to Jinapong et al. [15]: 100 ml of distilled water (at 21 °C) was poured into a beaker, a powder sample (0.1 g) was placed around the pestle (inside the funnel so that it blocked the lower opening) and lifted the pestle and started the stopwatch. Finally, time was recorded when the powder became completely wetted (visually assessed as the time when all the powder particles penetrated the surface of the water). The water adsorption kinetics was determined using a test bench that allowed the constant measurement and digital data storage. The change in the mass of the sample was measured over a 24 h period in an environment with a water activity of 0.338 and 0.648 (saturated salt solution) and at a constant temperature of 22 ± 1 °C [16].

All measurements were made at least two times. Results were expressed as mean ± standard deviations (SD). For the statistical analysis of the data, the ANOVA and regression analysis were used. Statistical differences were estimated using the LSD test, in relation to the applied variable using *F*-test. The statistical analysis was carried out by using the Statgraphics Plus 4.1 version software (StatPoint Technologes). The significance level was $p < 0.05$.

Results and discussion

In the study, β-LG – vitamin D3 complexes were synthesised. Fluorescence spectroscopy was applied to verify the binding of cholecalciferol to β-LG. The fluorescence of Trp 19, present in β-LG structure is quenched at the wavelenght of 332 nm in the case of ligand binding. The binding of cholecalciferol to β-LG resulted in the fluorescence quenching of the Trp at 332 nm. The fluorescence emission spectrum of the β-LG – cholecalciferol complex solution shifted to longer wavelenghts in relation to the β-LG solution without the ligand.

β-LG – vitamin D3 complexes with/without carbohydrates were characterised by similar values of water activity and the insolubility index (Table 1). Complexes with/without addition of carbohydrates, obtained by freeze-drying, were characterised by a larger particle size and better wettability in relation to spray-dried powders. Improved wettability was observed for β-LG – vitamin D3 complexes with the addition of lactose/trehalose (Table 1). The freeze-dried powders were characterised by wetting times lower than 15 s, which indicates that the complexes possess characteristics of "instant'' products (Table 1) [16].

The effect of the drying method and lactose/trehalose addition on the adsorption kinetics at a_w = 0.338 and a_w = 0.648 are shown in Fig. 1 and Fig. 2.

The powdered β-lactoglobulin – vitamin D3 complex (β-LG:D3) was very hygroscopic and adsorbed more water than the other powders studied. A significant difference occurred between the adsorption kinetics curves obtained for the complex with lactose (β-LG:D3:L) and trehalose (β-LG:D3:T), and those received for powders without additives (β-LG:D3). Loss of adsorbed water occurred within 24 h in β-lactoglobulin-vitamin D3 complexes with lactose or trehalose at a_w = 0.648, but not in complexes stored at a_w = 0.338. The loss of adsorbed water at a higher a_w value resulted from carbohydrates crystallization. Crystallization of carbohydrates is a time-dependent process which may occur from an amorphous state formed by freeze-drying and spray-drying (Kamrul Haque and Ross, 2004).

Table 1. Physical properties of β-lactoglobulin (β-LG) with vitamin D3 (D3) and lactose (L)/trehalose (T) complexes

Material	Water activity (-)	Particle size, μm	Insolubility index, ml	Wettability, s
Spray-dried β-LG:D3	0.244 ± 0.01^{a}	48.9 ± 4.7^{a}	0^{a}	31 ± 1^{d}
Spray-dried β-LG:D3:L	0.245 ± 0.01^{a}	54.5 ± 7.2^{a}	0^{a}	22 ± 5^{c}
Spray-dried β-LG:D3:T	0.247 ± 0.02^{b}	56.5 ± 7.1^{a}	0^{a}	20 ± 0^{c}
Freeze-dried β-LG:D3	0.244 ± 0.01^{a}	55.5 ± 3.6^{a}	0^{a}	15 ± 3^{b}
Freeze-dried β-LG:D3:L	0.246 ± 0.01^{ab}	80.2 ± 4.3^{b}	0^{a}	1 ± 0^{a}
Freeze-dried β-LG:D3:T	0.248 ± 0.02^{b}	75.0 ± 3.6^{b}	0^{a}	1 ± 0^{a}

[a] Values followed by different letter in the same column are significantly different at $p < 0.05$.

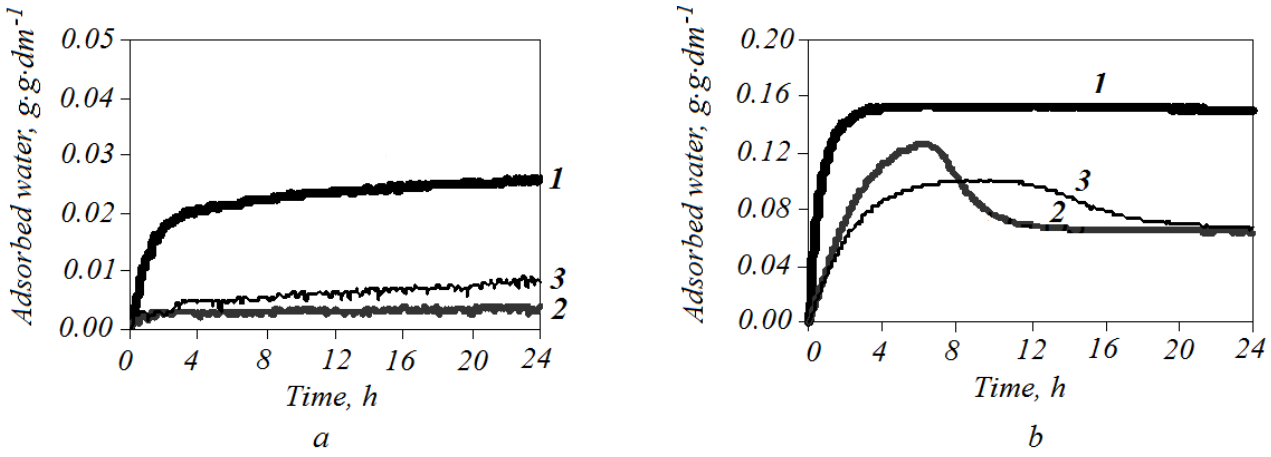

Fig. 1. Effect of spray-drying on adsorption kinetics of β-lactoglobulin (β-LG) with vitamin D3 (D3) and lactose (L)/trehalose (T) complexes: *a*) a_w = 0.338; *b*) a_w = 0.648. *1* – β-LG:D3; *2* – β-LG:D3:L; *3* – β-LG:D3:T

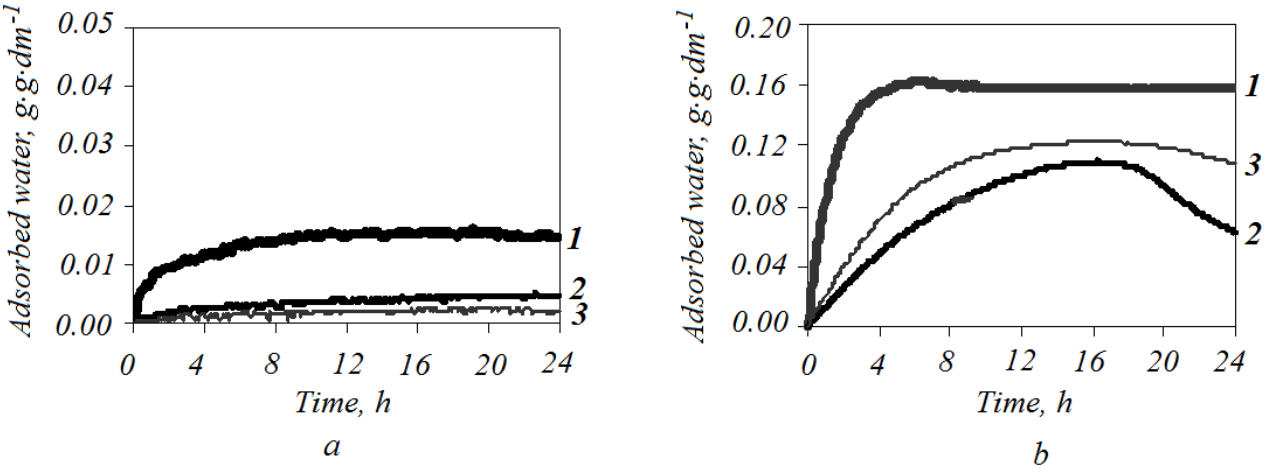

Fig. 2. Effect of freeze-drying on the adsorption kinetics of β-lactoglobulin (β-LG) with vitamin D3 (D3) and lactose (L)/trehalose (T) complexes: *a*) a_w = 0.338; *b*) a_w = 0.648. *1* – β-LG:D3; *2* – β-LG:D3:L; *3* – β-LG:D3:T

The crystallization of trehalose in a powdered complex (β-LG:D3:T) was found to be delayed in relation to the β-LG – vitamin D3 complex with lactose (β-LG:D3:L) (Fig. 1b and Fig. 2b). Water is released when the hygroscopic amorphous carbohydrates crystallize into the less hygroscopic crystalline forms (Kamrul Haque and Ross, 2004; Thomas, Scher, Desorby, 2005). The longer time to trehalose crystallization than to lactose crystallization in β-lactoglobulin – vitamin D3 – carbohydrate complexes could result from the higher amount of water required to form crystalline trehalose dihydrate than to form crystalline lactose monohydrate or anhydrate at limited sorbed water contents and the higher solubility and lower amount of available crystalline forms of trehalose, what is in agreement with researches provided by Zhou and Roos (2011).

Conclusions

1. Powdered complexes of β-LG – vitamin D3 with addition of lactose/trehalose, obtained by spray-drying and freeze-drying, were characterised by a larger particle size, very good wettability and reduced hygroscopicity as compared to powders without addition of carbohydrates.
2. β-LG – vitamin D3 complexes with/without addition of carbohydrates, obtained by freeze-drying, were characterised by a larger particle size and a better wettability as compared with spray-dried powders.
3. The stability of amorphous powders depends on material composition and storage conditions. The results showed that the addition of carbohydrates, lactose or trehalose had a similar water sorption but different crystallization properties. In the case of β-LG – vitamin D3 complexes, the crystallization of carbohydrates, especially trehalose crystallization, was delayed.

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K. Szulc, A. Górska

DŽIOVINTO β-LAKTOGLOBULINO – CHOLEKALCIFEROLIO KOMPLEKSO FUNKCINĖS SAVYBĖS

S a n t r a u k a

Šio tyrimo tikslas – nustatyti džiovinimo būdo įtaką β-laktoglobulino – cholekalciferolio komplekso (β-LG – vitaminas D3) su angliavandenių priedu fizinėms savybėms. Sausųjų β-LG – vitamino D3 kompleksų su angliavandenių priedu buvo geresnis drėgstamumas ir mažesnis higroskopiškumas, palyginti su mėginiais, gautais be angliavandenių priedo. Sausi miltelių pavidalo β-LG – vitamino D3 kompleksai su laktozės ir trehalozės priedu ir be jo, išdžiovinti sublimacinėje džiovykloje, turėjo didesnes daleles ir geriau drėko, nei purkštuvinėje džiovykloje gauti miltelių pavidalo β-LG – vitamino D3 kompleksai. Amorfinių miltelių stabilumas priklauso nuo medžiagų sudėties ir laikymo sąlygų. Tyrimo rezultatai parodė, kad pridėjus angliavandenių (laktozės ar trehalozės) buvo gaunama panaši įtaka kompleksų vandens sorbcinėms savybėms, tačiau skirtingai buvo veikiamos kristalizacijos savybės. Angliavandenių, ypač trehalozės, kristalizacija buvo uždelsta.